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EXAMINER	
HILL, KEVIN KAI	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/624,631	GRASSO ET AL.	
	Examiner	Art Unit	
	KEVIN K. HILL	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 February 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *19-25,27,28,72 and 73* is/are pending in the application.

4a) Of the above claim(s) *21-25 and 27* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *19,20,28,72 and 73* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

Election/Restrictions

Applicant has elected the invention of Group III, claims 19-35 and 42-49, drawn to methods for modulating antibody production of cells.

Within Group III, Applicant has further elected the restricted subgroup "f", regarding the molecular mutation/composition to inactivate the expression of the target gene product, as recited in claim 28, drawn to a knock-out mutation to disrupt the endogenous function of the target gene.

Within Group III, Applicant has further elected the method step inventive group "B", claims 35 and 44-46, drawn to a method for enhancing antibody production of cells comprising steps to inactivate or impair expression of the alpha-1-antitrypsin gene product.

Within Group III, Applicant has elected the high-titer antibody producing cell type species "hybridoma", as recited in Claim 20.

Amendments

In the reply filed February 28, 2008, Applicant has cancelled Claims 1-18, 26, and 29-71, withdrawn Claims 21-25 and 27, amended Claims 19 and 27-28, and added new claim, Claim 73.

Claims 21-25 and 27 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Claims 19-20, 28 and 72-73 are under consideration.

Priority

Applicant's claim for priority under 35 U.S.C. 119(e) regarding the parent provisional application 60/397,027, filed on July 19, 2002 is acknowledged. Accordingly, the effective priority date of the instant application is granted as July 19, 2002.

Information Disclosure Statement

Applicant has filed an Information Disclosure Statements on November 14, 2007 and May 5, 2008 that have been considered. The signed and initialed PTO Forms 1449 are mailed with this action.

A second copy of the Information Disclosure Statement on April 18, 2007 is provided with this action to correct the oversight of not having initialed the cited references, as requested

by Applicant. The Examiner notes however, that these references were considered at the time of the prior Office Action.

Examiner's Note

Unless otherwise indicated, previous objections/rejections that have been rendered moot in view of the amendment will not be reiterated. The arguments in the February 28, 2008 response will be addressed to the extent that they apply to current rejection(s).

Claim Objections

1. **Claim 28 is objected to because of the following informalities**: the claim is missing the word "encoding" on line 4, e.g. "...the function of a gene encoding alpha-1-anti-trypsin". See, for example, lines 5-6. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the Applicant regards as his invention.

2. **Claims 19-20, 28 and 72-73 are rejected under 35 U.S.C. 112, second paragraph**, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

As a first matter, a broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP § 2173.05(c). Note the explanation given by the Board of Patent Appeals and Interferences in *Ex parte Wu*, 10 USPQ2d 2031, 2033 (Bd. Pat. App. & Inter. 1989), as to where broad language is followed by "such as" and then narrow language. The Board stated that this can render a claim indefinite by raising a question or doubt as to whether the feature introduced by such language is (a) merely exemplary of the remainder of the claim, and therefore not required, or (b) a required feature of the claims. Note also, for example, the decisions of *Ex*

parte Steigewald, 131 USPQ 74 (Bd. App. 1961); *Ex parte Hall*, 83 USPQ 38 (Bd. App. 1948); and *Ex parte Hasche*, 86 USPQ 481 (Bd. App. 1949). In the present instance, claim 19 recites the broad recitation "a cell" (lines 1 and 6), and the claim also recites "mammalian cell" (line 4) which is the narrower statement of the range/limitation, wherein the mammalian cell may be a hybridoma (claim 20) or a rodent cell (claim 72). The breadth of the claimed cell (line 1) reasonably embraces an enormous genus of prokaryotic and eukaryotic cell types, and the relationship between the two cell types as it pertains to the method of producing high titer antibody producing cells is not clearly established.

As a second matter, Claim 19 recites the limitation "the cell" (line 6). There is insufficient antecedent basis for these limitation in the claims because, as noted above, it is unclear whether "the cell" is intended to be the high titer antibody producing cell (line 1) or the mammalian cell subject to the steps of genetic modification (lines 4-6).

As a third matter, Claims 20 and 72 recite the limitation "hybridoma cell" and "rodent cell" in reference to "the cell" of Claim 19. There is insufficient antecedent basis for these limitation in the claims because, as noted above, it is unclear whether the "hybridoma cell" and "rodent cell" are intended to be the high titer antibody producing cell (line 1) or be subject to the steps of genetic modification (lines 4-6).

As a fourth matter, Claim 19 (and dependent claims) are vague and indefinite in that no step(s) in the claimed method refers back to or recapitulates the preamble of the claim. Applicants recite a method of producing a high titer antibody producing cell, but no step is recited that actually accomplishes the preamble. The nexus between the method of producing a high titer antibody producing cell (line 1) and the steps of genetically modifying a mammalian cell (lines 4-6) is not clearly established in the claim. That is to say, the claim does not establish that the disruption of a gene encoding alpha-1-anti-trypsin (AAT) and/or a gene encoding endothelial monocyte activating polypeptide I (EMAP) are causal determinants for producing a high titer antibody producing cell. Rather, genomic knockout of AAT and/or EMAP may simply be a means of identifying cells from a population and have nothing to do with enhanced antibody production. Thus, it is unclear if additional, undisclosed steps are a part of, and directly responsible for, the claimed method of producing a high titer antibody producing cell rather than

inactivation of AAT and EMAP and therefore the metes and bounds of the claimed subject matter are unclear.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. **Claims 19-20, 28 and 72 stand and Claim 73 is newly rejected under 35 U.S.C. 112, first paragraph**, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

This rejection is maintained for reasons of record in the office action mailed August 29, 2007 and re-stated below. The rejection has been re-worded slightly based upon Applicant's amendment filed February 28, 2008.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention. If not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2ds 1400, 1404 (Fed. Cir. 1988)). Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not disclosed in the specification. Therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention. And thus, skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The Breadth of the Claims and The Nature of the Invention

The claimed invention is directed to an *in vitro* method for producing a high titer antibody producing cell comprising modulating the expression of at least one gene involved in antibody production.

The breadth of the claims reasonably embraces an enormous genus of high titer antibody producing cells encompasses diverse organisms across the prokaryotic and eukaryotic kingdoms, as Applicant has contemplated bacteria, yeast, plants and mammals, for example (page 4,

[0010]). The preferred embodiment of the instantly elected invention is a hybridoma cell (Claim 20) and a rodent cell (Claim 72). The claims are broad for encompassing an enormous genus of rodents in the animal kingdom. The art teaches that there are approximately 4,000 rodent species, divided into three major groups or sub-orders, Sciuromorpha, Myomorpha and Hystricomorpha, and more than 30 families. The diversity of instantly claimed rodent genus reasonably encompasses, for example, squirrels, chipmunks, beavers, woodchucks, prairie dogs, hamsters, lemmings, voles, porcupines, capybaras, agoutis, chinchilla, as well as many species whose common names include the term "rat" (columbia.thefreedictionary.com/rodent). It is unclear how the instantly claimed method, specifically using gene knock-out technology, may be performed in cell types that, by definition, do not encode AAT or EMAP genes, e.g. bacteria and yeast, for example.

When the claims are analyzed in light of the specification, the inventive concept of the instant application is to administer dominant-negative molecules, antisense molecules, ribozymes, knock-out targeting vectors, catalytic antibodies, polypeptide inhibitors, intracellular and/or extracellular antibodies, pharmacologic saturation of substrates or ligands, and molecules of biological or chemical basis that can affect the gene expression profile (page 4, [0012-0013]; page 7, [0022]) to modulate the expression of a gene involved in antibody production. The preferred embodiment of the instantly elected invention, Claim 28, is to suppress the expression of said gene(s) by introducing into the cell a knock-out targeting vector to disrupt the function of said gene(s). The inventive concept of the instant application is to inhibit or disrupt the expression of alpha-1-antitrypsin (AAT) gene expression and/or function and/or endothelial monocyte activating polypeptide I (EMAP) gene expression and/or function to effect high-titer antibody producing cells (page 7, [0022]). The preferred embodiment of the instantly elected invention, Claim 28, is to introduce into the cell a knock-out targeting vector to disrupt the function of genes encoding alpha-1-antitrypsin (AAT) and/or endothelial monocyte activating polypeptide I (EMAP).

The Amount of Direction Provided by the Inventor and The Existence of Working Examples

The inventive concept of the instant application is a method to inhibit or disrupt the function of alpha-1-antitrypsin (AAT) and/or endothelial monocyte activating polypeptide I (EMAP) genes to effect high-titer antibody producing cells (page 7, [0022]). The preferred embodiment of the instantly elected invention, Claim 28, is a method to suppress the expression of said gene(s) by introducing into the cell a knock-out targeting vector to disrupt the function of said gene(s). Given the absence in the prior art teaching a mechanism linking AAT and EMAP expression/activity and antibody production, an artisan is dependent upon the instant disclosure to provide specific, not general, guidance.

With regard to the method for enhancing antibody production in a hybridoma cell by introducing a knock-out vector into the cell to inactivate expression of genes encoding AAT and/or EMAP, the specification does not teach an example of the claimed method. Rather, the specification teaches that the H6 hybridoma cell line containing vectors expressing structurally unknown antisense constructs to both the alpha-1-antitrypsin (AAT) gene of SEQ ID NO:1 and the endothelial monocyte-activating polypeptide I (EMAP) gene of SEQ ID NO:2 (page 24, Example 4, Table 2). However, given the absence of a structural disclosure identifying the specific chemical nature of these antisense constructs, the degree of unpredictability in the art

regarding antisense RNA methods to decrease the expression of one's gene(s) of interest, the fundamental nature of numerous AAT genes existing in the hybridoma cell, and the novelty of the observed cellular response, one of ordinary skill in the art cannot reasonably predict the phenotype obtained when the individual gene(s) is(are) totally disrupted.

The specification discloses that the enhanced antibody production phenotype is obtained when both AAT and EMAP I activities are suppressed. The question then arises whether both activities must be suppressed to yield the desired phenotype or if only one activity is necessary. Applicant discloses having used AAT antiserum that recognizes the peptide QSPIFVGKVVDPTHK (SEQ ID NO:17) to corroborate the antisense experiment (pg 25, Examples 5-6), and was successful in measuring increased antibody production, thus indicating that suppressing EMAP activity is not necessary *a priori*.

The Examiner notes that the peptide QSPIFVGKVVDPTHK is present (100% identical) in the murine A1AT2 (NP-033270.3) and A1AT4 (NP-033272.1) genes, substantially present (one amino acid difference) in murine A1AT1 (NP-033269.1), A1AT3 (NP-033271.1), A1AT5 (NP-033273.1) and A1AT6 (P81105) genes, and substantially present (three amino acid differences) in the human A1AT gene (see Search Results). Thus, the antiserum experiment does not shed light on the question of which specific AAT protein(s) is responsible for the observed phenotype because up to six murine and one human A1AT polypeptides may have been functionally inactivated by the antiserum.

The specification does not disclose if suppression of EMAP activity alone is sufficient to yield the increased antibody production phenotype. Given that mice encode two distinct genes, NP-058020.1 on chromosome 3 and XP-982480.1 on chromosome 18, the question of functional redundancy raised for AAT is also germane for EMAP I. The specification is silent with evidence demonstrating that inactivation of EMAP alone by antisense or antiserum can also recapitulate the increased antibody production phenotype. The Examiner notes that the antiserum experiment of the instant specification would not address the issue of functional redundancy because both murine polypeptides are recognized by the antiserum directed against the EMAP peptide (pg 26, line 1, SEQ ID NO:18).

The scope of high titer antibody producing cells (claims 19 and 28) encompasses diverse organisms across the prokaryotic and eukaryotic kingdoms, as Applicant has contemplated bacteria, yeast, plants and mammals, for example (page 4, [0010]). It is unclear how the instantly claimed method, specifically using gene knock-out technology, may be performed in cell types that, by definition, do not encode AAT or EMAP genes, e.g. bacteria and yeast, for example. The artisan cannot remove a gene that is not present *a priori* in a given cell and reasonably expect to enhance antibody production in said cell.

The State of the Prior Art, The Level of One of Ordinary Skill, and The Level of Predictability in the Art

The art teaches (Forsyth et al, 2003) that alpha-1-antitrypsin (AAT), also known as alpha-1-protease inhibitor, is a member of the rather large (>700 known to date) and functionally diverse family of serine protease inhibitors (SERPINs). Forsyth et al teach that, in striking contrast to the human and bovine genomes wherein AAT is represented by a single gene, four genes are found in the guinea pig and rabbit and individual mouse species possess as many as five AAT genes (page 337, column 2). Furthermore, intraspecific gene number variation is

observed, as the *M. domesticus* C57/BL/6J, AJ, and C3H/HeJ laboratory strains express five AAT variants; whereas, the *M. domesticus* AKR/J and DBA/2J laboratory strains express only three AAT variants. Each member of the AAT gene family shares the strongly conserved, protease recognition region that is exposed for interaction with the AAT ligand. Thus, numerous AAT genes exist in the disclosed H6 hybridoma cell line, including other mammalian AAT gene(s), the Applicant-disclosed murine AAT gene of SEQ ID NO:1, and at least two and potentially four, additional ATT murine genes.

With respect to any effect alpha-1-antitrypsin (AAT) activity contributes towards antibody production, the art is largely silent. Jeanin et al teach that exogenously applied AAT potentiates IgE and IgG4 synthesis in human peripheral blood morphonuclear cells (PBMCs) and B-cells, resulting in a 950% increase in IgE production (see Figures 1-3 and Table 1). In contrast to the *trans*-effect taught by Jeanin et al, the art makes no mention of any *cis*-effect AAT expression, or lack thereof, contributes towards antibody production within the given host cell wherein AAT gene expression has been abrogated, down-regulated or disrupted entirely. Rather, the art teaches that the build-up of improperly folded AAT contributes to human disease by impairing protein maturation in the endoplasmic reticulum, polymerizing into protein aggregates and forming intracellular inclusion bodies, and ultimately causing cytotoxicity (Welch et al, 2004). Thus, complete removal of the alpha-1-antitrypsin (AAT) gene(s) in any organism for the production of high-titer antibody producing cells, as disclosed in the specification, is not a routine practice, and despite the general high level of expertise in the art, considerable unpredictability exists in the field regarding any effect AAT has on antibody production and secretion, especially in light of the organism-dependent diversity of AAT gene number.

Endothelial monocyte-activating polypeptide I (EMAP I) is also known in the art as S100 calcium binding protein A11 (S100A11) and Calgizzarin (Marenholz et al, Biochem. Biophys. Res. Comm. 322: 1111-1122, 2004; pg 1113, Table 1). Santamaria-Kisiel et al (Biochem J. 396:201-214, 2006) teach that the S100 protein family comprises at least 25 members, forming the largest group of EF-hand signaling proteins in humans (Abstract; pg 202, col. 1, S100 proteins). S100 proteins are proposed to have intracellular and extracellular roles in the regulation of many diverse processes such as protein phosphorylation, cell growth and motility, cell-cycle regulation, transcription, differentiation and cell survival (pg 203, col. 1, Biological Roles), wherein S100A10, A11 and A16 have broad tissue distribution (Marenholz et al, pg 1117, col. 2, last ¶).

The murine Calgizzarin gene (a.k.a. S100A11) has been knocked out, and the resulting null mice display no obvious phenotypes (Mannan et al, Molecular Reproduction and Development 66: 431-438, 2003). Mannan et al hypothesize that the lack of a phenotype may be due to functional redundancy or compensation by another member of the S100 gene family (pg 437, col. 1, ¶1), wherein a minor increase in protein level by the hypothesized S100 family member could significantly influence the ability of said family member to function as a mediator in calcium signaling pathways, thereby compensating for the loss of S100A11. Mice encode two distinct genes. NP-058020.1 (S100A11) is located on chromosome 3 and is 100% identical to U41341.1 disclosed in the instant specification. XP-982480.1 is located on chromosome 18. XP-982480.1 was identified in the art quite recently, June 20, 2007, and thus there is no evidence for

or against the ability of this essentially identical polypeptide to functionally compensate for S100A11. However, given the striking, nearly identical amino acid sequences between S100A11 and XP-982480.1, differing from S100A11 by one amino acid change, $G_{53} \rightarrow D_{53}$, which lies outside of the Helix I-Helix II and the Helix III-Helix IV S100 and Canonical EF-hands, respectively, it would not be surprising if the two polypeptides are functionally equivalent.

The art teaches that hybridoma technology for the production of antibodies for both research and therapeutic purposes has been in use for almost thirty years, and thus the level of ordinary skill in the art is high (Laffly and Sodoyer, 2005). Laffly and Sodoyer recognize that "the huge demand for large amounts of monoclonal antibodies is currently driving improvement of existing expression systems or the quest for alternative production means" (page 45, column 2, lines 4-7). The art recognizes that the term "hybridoma", as commonly used in the art, represents antibody producing B-cells fused with immortalized myeloma cells, wherein the mammalian species of the B-cells and the mammalian species of the myeloma cells need not be identical, to produce a rapidly and indefinitely growing population of hybridoma cells that will produce antibodies, wherein each specific hybridoma fusion will produce one type of antibody. The specification does not disclose the species derivation of the H6 hybridoma cell; however, Komori et al (1988), for example, teach a human-mouse hybridoma (H6-3C4). The specification does not provide any disclosure regarding the number of existing mammalian AAT genes expressed within the H6 hybridoma cell line.

At the time of filing, the art did not consider the phenotype of a knock-out to be predictable. The art teaches that while the promise of gene targeting had been to reveal the *in vivo* function of a gene of interest, the functional relevance of gene targeting has been questioned because the mutation might lead to an avalanche of compensatory processes (up- or down-regulation of gene products) and resulting secondary phenotypical changes. The art recognizes (Doetschman, 1999) that knock-out targeting vectors require the presence of DNA sequences homologous to the target gene and flanking the disrupted gene cassette to facilitate homologous recombination into the targeted genome, thus replacing the endogenous gene with an exogenous gene fragment and thereby altering the endogenous gene in a pre-specified manner. Doetschmann provides numerous examples of instances in which genes considered well-characterized *in vitro* have produced unexpected phenotypes or indiscernible or no phenotypes in transgenic or knockout mice. Moens et al (1993, Development, 119: 485-499) further teach that different mutations in the same gene can lead to unexpected differences in the phenotype observed. Moens et al. shows that two mutations produced by homologous recombination in two different locations of the *N-myc* gene produce two different phenotypes in mouse embryonic stem cells, one leaky and one null. A null mutant organism might not only lack the product of a single gene, but might also possess a number of physiological or other processes that have been altered to compensate for the effect of the null mutation (Gerlai, 1996, Trends Neurosci, 19: 177-181, page 177, column 1, paragraph 1). Gerlai teaches an example wherein background genotype can confound the exhibited phenotypes. Targeted disruption of a gene of interest, Gene 1, might lead to changes in expression of alleles b and B for Gene 2. A regulatory change in Gene 2 might lead to different phenotypic changes, depending on which allele (b or B) is present in the organism with the null mutation in Gene 1. The consequences of this problem is that due to this

polymorphism in the genetic background, one cannot conclude for certain that a phenotypic change exhibited in a null-mutant resulted from the null mutation or to the genetic background (Gerlai, page 177, column 1, under "Polymorphism in the genetic background might make the results of gene-targeting studies difficult to interpret").

Thus, the art at the time of filing clearly establishes the unpredictability of determining the phenotype of transgenic or knockout conditions even when the activity of the gene has been extensively studied *in vitro*. With respect to the instant invention, Gerlai's teachings indicate that an artisan cannot predict that any gene disruption would necessarily result in a phenotype. And if a phenotype were to result, an artisan cannot predict that the resultant phenotype was the result of the gene disruption. The teachings by Gerlai indicate that phenotypes exhibited by knock-out transgenes can be the result of unrelated factors. The teachings in the art indicate that guidance needs to be given such than an artisan knows how to discriminate what phenotypes are the result of AAT and/or EMAP gene disruption(s) and what are the result of non-specific factors such as genetic background. Given that alpha-1-antitrypsin (AAT) is a member of the rather large genus of serine protease inhibitors (SERPINS) that have a variety of diverse functions in the animal, an artisan cannot predict what biological function occurs in a family of SERPINS such that an artisan would know that the phenotype exhibited in the knockout cell or organism is a result of the gene disruption. The artisan cannot reasonably predict that the phenotype is the result of gene disruption, because the art teaches that non-specific factors, such as genetic background, affect the presence or absence of phenotypes. As such, the specification fails to teach that the phenotypes have a biological relationship with AAT and/or EMAP gene disruption.

With regard to the ability of an artisan to correlate an observed antisense RNA phenotype to a predicted phenotype using targeting vectors that knock-out a targeted gene, Caplen teaches that the RNAi machinery can be saturated, so there will probably be a limit to the number of different genes that can be targeted in a cell at one time (page 1244, column 1). Furthermore, Caplen expresses the importance in recognizing that there is variation in the degree of inhibition mediated by different small interfering RNA sequences which may result in the production of different phenotypes. Thus, the disclosure of a phenotype in response to the expression of a single, structurally undefined antisense molecule (page 24, Example 4, Table 2, discussed below) cannot reasonably predict the phenotype obtained when the individual gene is totally disrupted.

The Quantity of Any Necessary Experimentation to Make or Use the Invention

The substantive technical issue with the instant invention is the realistic possibility of functional redundancy between the related murine and human A1AT family members and the (at least two) murine and human S100A11 family members extant in the murine genome if murine cells (alone or as hybridoma) are to be used in the claimed invention. In the absence of disclosure regarding the structural nature of the antisense oligonucleotides and what specific gene(s) said oligonucleotides inactivate, the artisan would essentially have to knockout by gene targeting five murine A1AT genes, a potential sixth murine A1AT gene (A1AT6), two murine EMAP genes, and in the case of a murine/human hybridoma, an additional human A1AT gene and S100A11 gene. Thus, ten distinct genes must be inactivated by targeted gene disruption. Furthermore, because each genome is diploid, both alleles of each gene must be disrupted, thus requiring twenty independent knockout events because the specification does not teach how to predictably select for cells that are homozygous for each particular AAT and EMAP/S100 gene that has been

disrupted. While the art teaches that sequential targeted disruption, e.g. triple knockout, is possible (Robinson et al, PNAS 96: 11452-11457, 1999), the art is silent with respect to generating twenty genomic knockout events in a single cell. The Examiner notes that Robinson et al created triple knock-out animals by starting with embryonic stem cells already heterozygous deficient for two of the desired genes, making the third knockout mutation, creating heterozygous triple knockout animals and obtaining homozygous triple knockout cells via mating. The instant invention is drawn to cells cultured *in vitro*, e.g. hybridoma, and thus a selection scheme must be set forth to predictably select for homozygosity of each of the ten AAT and EMAP/S100 genes that are to be knocked out.

Applicant argues that as noted by the Forsyth et al (Genomics, 81:336-345, 2003), the SERPIN family to which the AAT genes belong are functionally diverse, and thus there is no basis for concluding that various AAT genes are functionally redundant other than structural homology, and also that there is no basis for structurally similar EMAP genes being functionally redundant (Arguments filed February 28, 2008; pg10 of 12). However, if Applicant should take such a position, then it follows that the claims lack enablement because neither the claims nor the specification clearly identify which specific AAT family member of the functionally distinct AAT genes, and which specific EMAP I family member of the functionally distinct EMAP I genes, is responsible for the claimed phenotype.

Furthermore, Applicant's argument also embraces the position that the human AAT gene is not considered to be functionally homologous to any one of the plurality of murine AAT genes, and that the human EMAP I gene is not considered to be functionally homologous to any one of the murine EMAP I genes, as such genes would likely also be functionally distinct from the corresponding murine genes. Thus, it would follow that the claims lack enablement because knockout mutations in the enormous genus of mammalian cell types, nor enormous genus of rodent cell types, would not, by definition, yield high titer antibody cells because Applicant's position is that the AAT and EMAPI genes extant in the other mammalian genomes are functionally distinct from the murine AAT gene, whichever one it is that Applicant functionally inactivated via that structurally undisclosed antisense RNA, so as to achieve high antibody titers. The bona fide AAT and EMAP I genes in the plurality of mammalian organisms that are functionally homologous to the plurality of murine AAT and EMAP I gene(s) responsible for modulating antibody titers has not been established in the instant specification or in the art.

However, if Applicant's position is that the human AAT and EMAP I genes are functionally homologous to the murine AAT and EMAP I genes, then it logically follows that the artisan would have to introduce knockout targeting vectors to the human AAT and EMAP I homologues as well as the murine AAT and EMAP I genes (minimally eight targeting events: two alleles for each of four genes) so as to overcome functional redundancy in a hybridoma cell and manifest high antibody titers.

Therefore, in view of the art recognized diversity in species-specific AAT gene number(s), the silence in the art teaching any role(s) any alpha-1 antitrypsin activity has on antibody production, the breadth of the AAT genes and disruptions in the AAT and/or EMAP/S100 genes claimed, the lack of specific demonstrations in the disclosure teaching method steps of introducing a targeting vector to disrupt the up to ten genes (murine/human hybridoma cell) encoding any AAT, alone or in combination with, EMAP/S100 that will cause increased antibody production, the lack of a null phenotype resulting in high titer antibody production observed by the prior art when AAT and/or EMAP genes are knocked-out in cells or organisms, the unpredictability in determining a knock-out phenotype even when the activity of the gene has been *extensively* (*emphasis added*) studied *in vitro*, the unpredictability in correlating any observed phenotype in a knockout cell or organism with gene disruption as acknowledged by the prior art, and the etiological and pathological diversity of hypoimmunoglobulin disease origins, the quantity of necessary experimentation to make or use the invention as claimed, based upon what is known in the art and what has been disclosed in the specification, will create an undue burden for a person of ordinary skill in the art to demonstrate that the method step of introducing a knock-out targeting vector to inactivate any alpha-1-antitrypsin gene(s), alone or in combination with, endothelial monocyte activating polypeptide I gene(s) in a cell will result in a high-titer antibody producing cell or enhance antibody production in a cell associated with hypoimmunoglobulin disease.

Applicant's Arguments

Applicant argues that:

a) The state of the art of gene knockout technology was highly developed and the level of those of skill in the art was quite high as of the priority date of the present application. Moreover, gene knockout technology was so advanced and routine that it was regularly taught and practiced in the laboratory portion of college biology courses. See Kline Declaration, paragraph 12. Once Applicants identified the significance of suppression of expression of the alpha-1-antitrypsin and endothelial monocyte activating polypeptide I genes on antibody production, it required no more than routine experimentation on the

part of the ordinarily skilled artisan to develop polynucleotides for introduction into antibody-producing mammalian cells to enhance antibody production in accordance with the claimed methods. It would have required no more than routine experimentation on the part of the ordinarily skilled artisan to design one or more knock-out targeting vectors directed to the alpha-1-antitrypsin and endothelial monocyte activating polypeptide I genes and to introduce the vector(s) in an antibody-producing mammalian cell to suppress gene expression in accordance with the claims in view of the knowledge and skill in the art and the detailed teachings of the present specification.

b) Applicant argues that as noted by the Forsyth et al (Genomics, 81:336-345, 2003), the SERPIN family to which the AAT genes belong are functionally diverse, and thus there is no basis for concluding that various AAT genes are functionally redundant other than structural homology. The Examiner's assertion of functional redundancy among members of the AAT and EMAPI gene families so as to undermine the predictability of the claimed methods is unsupported.

c) Applicant argues that the working examples, e.g. the microarray data revealing suppressed AAT and EMAP transcripts, antisense experiments directed against AAT and EMAP, thereby supporting the effect of suppression of expression of AAT and EMAP I on antibody production.

d) AAT and EMAP knockout would not be expected to be lethal. Indeed, mice null for the murine calgizzarin like gene were viable. Additionally, an antisense-knockdown phenotype generally correlates with its respective knockout phenotype. Thus, those of skill in the art would have reasonably expected that the AAT and EMAP antisense phenotypes would also be observed in cells in which these genes have been knocked out.

Applicant's argument(s) has been fully considered, but is not persuasive.

A) As has been discussed in the telephone interview (July 11, 2007) and prior Office Actions, the substantive issue is that Applicant has used a structurally undisclosed antisense RNA to inactivate an AAT and an EMAPI gene. However, Applicant has provided no evidence as to which one of the plurality of AAT and EMAP genes were inactivated so as to predictably give rise to a high titer antibody producing cell. Furthermore, while an antisense RNA molecule

may be capable of functionally inactivating more than one closely related AAT and EMAPI genes, respectively, the instantly elected invention is drawn to gene-targeted knock-out mutations, whereupon the other closely related AAT and EMAP family members may well be capable of functional compensation due to genetic redundancy. The instant specification does not disclose the specific AAT or EMAP gene must be inactivated to give rise to the claimed phenotype, nor how many other AAT and/or EMAP genes must also be inactivated to overcome functional compensation due to genetic redundancy. Thus, Applicant is essentially requiring the artisan to solve Applicant's problem, and for this reason, the instant invention is not "ready for patenting".

As for the technical ability to knock out a desired gene, Applicant is respectfully reminded that the invention as claimed requires that the artisan to knockout by gene targeting at least five murine A1AT genes, a potential sixth murine A1AT gene (A1AT6), two murine EMAP genes, and in the case of a murine/human hybridoma cell, an additional human A1AT gene and S100A11 gene. Thus, at least ten distinct genes must be inactivated by targeted gene disruption. Furthermore, because each genome is diploid, both alleles of each gene must be disrupted, thus requiring at least twenty independent knockout events because the specification does not teach how to predictably select for cells that are homozygous for each particular AAT and EMAP/S100 gene that has been disrupted. Neither the Kline Declaration nor the art teach how to generate twenty genomic knockout events in a single cell, thus such extensive genetic modification is not considered "routine".

B) While Serpin A1 family members may have different but overlapping in biological enzymatic activity as it pertains to substrates such as elastase, trypsin, chymotrypsin and/or cathepsin G, the respective Serpin A1 activities may still substantially and functionally overlap as it pertains to antibody production, as there is no evidence to exclude any one or more Serpin A1 family members as being not responsible for causing Applicant's observed increase in antibody production. Antibody production is a distinctly different functional assay than cleavage of elastase, trypsin, chymotrypsin and/or cathepsin G.

Those of ordinary skill in the art are aware of many examples of functional and genetic redundancy in biological systems, and the Examiner has set for sound technical and scientific

reasoning to support his or her conclusion that functional and genetic redundancy would prevent enablement of the instantly claimed method.

C) The obfuscation of the antisense experiment has been addressed in the enablement rejection above and said experiment does not clarify which AAT gene and which EMAP I gene must be inactivated to manifest high antibody titers. The microarray analysis and over-expression results are not commensurate in scope to the claimed method because they only disclose SEQ ID NO:1 and SEQ ID NO:2; whereas, the instant claims embrace many more genes besides those directly supported by the instant application.

D) Again, the distinction between the prior art and the claimed invention is the number of AAT and EMAP I genes that must be functionally inactivated by targeted genomic knockout so as to achieve high antibody titers. The loss-of-function of a single AAT gene, Calgizzarin, does not establish the viability or lethality for a cell in which most or all AAT genes have been knocked out, alone or in combination with most or all of the EMAP I genes have been knocked out. Applicant does not appear to be recognize the distinction between loss of function of a single gene versus the loss of function of a plurality of genes within a single cell/organism. Furthermore, as discussed above and in the telephone interview with Applicant's representative, the antisense experiment used in the specification's working example is structurally undisclosed, and there is no evidence that the AAT antisense is specific for a particular AAT gene, nor that the EMAP antisense is specific for a particular EMAP I gene. Thus, the antisense phenotype does not predictably correlate to the plurality of genomic knockout mutations as claimed.

Conclusion

4. No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Kevin K. Hill, Ph.D. whose telephone number is 571-272-8036. The Examiner can normally be reached on Monday through Friday, between 9:00am-6:00pm EST.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Joseph T. Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Kevin K. Hill, Ph.D./
Examiner, Art Unit 1633

/Q. JANICE LI/
Primary Examiner, Art Unit 1633